## PATENT SPECIFICATION

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## (54) A PROCESS FOR IMPROVING THE FUNCTIONAL PROPERTIES OF PROTEIN MATERIAL

We, STANDARD OIL COMPANY, a corporation organized and existing under the laws of the State of Indiana of 200 East Randolph Drive, Chicago, Illinois 60601, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:-

This invention relates generally to the improvement of the functional properties of proteinaceous materials such as single-cell proteins, plant proteins, whey solids, and mixtures thereof. More precisely, this invention involves subjecting the protein-containing material to a controlled pH, temperature, and time treatment which results in the improvement of the functional properties. For purposes of this invention, yeasts are considered as being separate from the plant proteins and are included within the single-cell protein category.

In recent years much attention has been directed toward the development of protein materials which can be incorporated in foods or food additives suitable for human consumption. Looking at plant proteins available today, it has been observed that these materials contribute to the off flavor, after flavor, undesirable color, unbalanced nutrients, or unacceptability in various food products. Similarly, untreated single-cell protein materials have been observed to have adverse effects on dough property and the bread quality. As would be expected, mixtures of singlecell and plant protein material have undesirably functional characteristics from

each of the separate protein source materials. The use of single-cell materials as a source for protein and the problems associated therewith can be better understood by looking more closely at a member selected from this class of materials, such as yeast cells. Yeast cells have the characteristic flavor and aroma which are affected to some extent by the growth conditions and the after-harvest processing conditions. They have a complicated organoleptic profile which consists of both pleasing and unpleasant flavors. One of the reasons limiting the use of yeast materials in food systems is the deleterious effect of its "yeasty" flavor. Where it is desirable to use yeast material at high levels for protein enrichment, a product of bland taste is preferred. Although the majority of yeasty flavor components can be easily removed from the yeast cells by a hot water extraction method, the use of such a process results in the loss of 15 to 20% in product yield. Furthermore, the extracted cells will retain some bitter, beany, and metallic off-taste. The loss in yield may be compensated by the value of the meat-flavored extract as a by-product, but the poor flavor of the cell product would need definite improvement. In addition, the hot water-extracted yeast cells contain about 0.6 to 1.0% phosphorus and 0.01 to 0.02% calcium. In order to achieve a nutritional balance of the calcium-phosphorus ratio for a food

Particular attention has been directed to the use of single-cell protein materials, such as yeast, as a replacer for egg solids and nonfat dry milk (NFDM). For example, in the bakery industry, 2 to 3% nonfat dry milk is normally used as an additive to improve the physical and nutritional quality of bread. However, in view of the increasing cost and decreasing availability of milk, many bakers are looking for a substitute. Although certain products derived from soy proteins have gained some acceptance, the active search by food technologists for a suitable substitute for milk in food products continues.

system in which such yeast is used, additional calcium may be necessary.

In this regard we have observed that during the fermentation and baking of bread dough, the wheat protein (gluten) forms the structure to hold the small



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	bubbles of gas which are generated. This functional property permits the bread to rise and results in the production of bread having good volume and fine crumb structure. However, when untreated single-cell materials, such as dried inactive	
5	yeast, are added to bread dough to replace 2% nonfat dry milk, undesirable changes are observed in the property of the dough which adversely affect the bread quality. Typically, dough which contains untreated yeast is soft, stringy, sticky and moist to the extent of rendering it difficult to handle. In fact, the dough has poor machinability characteristics which are detectable from the mixing to the final	5
10	proofing stage. The inferior property of the dough is probably due to the poor water absorption and the strong reducing property of the thiol group in the yeast cell which damages the gluten structure. We have now found that materials such as yeast plant, whey solids and combinations thereof can be treated according to the	10
15	process of this invention to yield products highly suitable for replacing egg solids and nonfat dry milk. During the treatment of the yeast cells in accordance with the present process, several things happen which improve the functional property of the cell. The yeasty off-flavor is greatly reduced and cell material becomes significantly bland in taste by heating the yeast cells under controlled pH reaction conditions. A large amount of buffering materials is released from the cell by the	15
20	heating process, which increases the buffering capacity of the food system when they are incorporated as dry yeast cell material. The saponification of lipid material gives rise to a soap material which is a good emulsifier. Also, heating under alkaline pH conditions will enhance the auto-oxidation of the thiol groups and the water holding capacity.	20
25	According to this invention, there is provided a process for treating protein materials selected from single-cell protein material, plant protein material, whey solids, or mixtures thereof in a manner whereby the color, flavor, nutritional value, and functional properties of said materials are improved for food use.  According to the present invention there is provided a process for improving	25
30	the functional properties of protein-containing materials comprising the steps of:  (a) preparing an aqueous slurry of a protein-containing material selected from  (1) single-cell protein, (2) plant protein, (3) whey material, and (4)  mixtures of single-cell protein with plant protein, whey solids, or both plant protein and whey solids, said mixtures containing from 1 to 99	30
35	weight percent of the single-cell protein component;  (b) heating the aqueous slurry to a temperature of from 75° to 100°C.;  (c) adjusting the pH of the heated slurry to within the range of 6.6 to 8.0 by adding a compound selected from anhydrous ammonia, ammonium hydroxide, calcium hydroxide, sodium hydroxide, sodium bicarbonate,	35.
40	calcium sulfate, potassium carbonate, calcium carbonate, sodium carbonate, potassium hydroxide, magnesium hydroxide and mixtures thereof;  (d) maintaining the heated, pH-adjusted slurry at said conditions for a time period of from 1 to 120 minutes; and	40
45	(e) drying the material from step (d).  In carrying out the process of the invention the aqueous slurry is treated with a basic compound, preferably a calcium compound, and may be fortified with an amino acid such as methionine or cystine. An aqueous slurry of the protein material is prepared and heated to a temperature of from 75° to 100°C, and the pH	45
50	of the heated protein material is adjusted within the range of 6.6 to 8.0, preferably 7.2 to 7.6, by adding a pH adjusting compound. The pH adjusting compound can be selected from anhydrous ammonia, ammonium hydroxide, calcium hydroxide, sodium hydroxide, sodium bicarbonate, calcium sulfate, potassium carbonate, calcium carbonate, sodium carbonate, potassium hydroxide, magnesium	50
55	hydroxide, and mixtures thereof, especially mixtures of calcium hydroxide and calcium carbonate or calcium sulfate. Additionally, the pH adjustment can be accompanied by the agitation and oxidation of the single-cell protein. The pH adjusted solution is maintained at temperature for a period of 1 to 120 minutes, preferably 1 to 10 minutes and most preferably about 2 minutes and then dried.	55
60	Alternatively the pH adjusted slurry, after having been maintained at the aforementioned conditions of pH and temperature for from 1 to 120 minutes, is separated into (1) a protein extract and (2) a base-treated protein material, particularly with a basic calcium compound, wherein the base-treated protein material is removed, water washed and dried with or without the addition of amino	60
65	acids. The protein extract can be heated to an increased concentration and dried for use as a seasoning ingredient.	65

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	By the practice of this invention one can prepare a proteinaceous material having improved functional properties from single-cell protein, plant protein, whey solids, or mixtures thereof.	
	It is believed that any microbial cell material, plant protein, whey solution, or	
5	mixtures thereof can be treated according to the process of this invention, although	5
-	this invention is particularly suited for processing yeasts such as Candida utilis. In a	
•	fully integrated, continuous system, microbial cells are conveniently grown in a	
	first fermenting stage where oxygen and a suitable substrate, such as liquid or	
10	gaseous hydrocarbons or oxygenated hydrocarbons such as carbohydrates or alcohols, together with a nutrient solution containing minerals are fed to a stirred	10
	reactor containing the microorganisms. In a continuous fermentation at steady	.0
	state, a portion of the reacting mixture is withdrawn at a constant concentration of	
	microorganisms. The concentration of the cells is typically increased by mechanical or evaporative means. As the microorganism concentration increases,	
15	a portion of the reacting mixture is withdrawn from the stirred reactor and the	15
••	microorganisms are separated from the withdrawn reaction mixture.	1.5
	By way of illustration, bacteria such as those listed in Table I, yeasts such as	
	those listed in Table II, and fungi such as those listed in Table III are suitably single-cell protein materials for use as starting materials in the practice of this	
20	invention.	20
		20
	TABLE I	
	Suitable Bacteria	
	Acetobacter sp. Arthrobacter sp.	
25	Bacillus subtilis	25
	Corynebacterium sp.	
	Micrococcus sp.	
	Pseudomonas sp.	
	TABLE II	. 30
30	Suitable Yeasts	30
	Candida curvata Candida lipolytica	
	Canalaa uposynca Candida pulcherima	
• •	Candida utilis	
35	Hansenula anomala	35
	Pichia farinosa Oidium lactis	
	Saccharomyces carlsbergensis	
40	Saccharomyces cerevisiae	40
40	Saccharomyces fragilis	40
	Trichosporon cutaneum	
	TABLE III Suitable Fungi	
45	Aspergillus niger Aspergillus glaucus	45
-	Aspergillus oryzae	
	Aspergillus terreus	
	Aspergillus itaconicus Penicillium notatum	
50	Penicillium chrysogenum	50
-	Penicillium glaucum	
	Penicillium griseofulyum	
	Candida utilis, Saccharomyces cerevisiae, Saccharomyces fragilis, ot	
	Saccharomyces carlsbergensis are suggested single-cell starting component materials	
55	for the process of this invention, because each is approved by the U.S. Food and Drug Aministration as suitable for use in food products.	55
	The plant protein material is advantageously selected from oil seed protein	
	materials such as soy flour, defatted soy flour, soy flakes, soy protein isolates and	
	concentrates, cotton seed flour, cotton seed protein isolates and concentrates,	
60	neanut flour neanut protein isolates and concentratess sesame seed flour sesame	60

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	concentrates, gluten, cereal proteir	trates, corn grits, corn protein isolates and isolates and concentrates, rapeseed flour and		
5	rapeseed protein isolates and concentrates.  The whey material can be whey solids in the form of an aqueous solution, condensed suspension of crystals, or a dried powder. The whey may be derived from the processing of Cheddar, Brick, Edam, Parmesan, Gouda, Emmenthaler			
10	Examples I to XI are illustrative, v	diagrams (Figures 1 to 3), Tables IV to VIII, and without implied limitation, of this invention. In wise specified, all percentages are by weight.	10	
	The following three testing san slurry under the conditions as des	EXAMPLE I nples were prepared from a 10% solids yeast cell cribed in the diagram shown in Figure 1.		
15	(a) untreated spray-dried cells (b) heated at 95°C. and pH 5.9 for 30 minutes (c) heated at 95°C. and pH 7.5 (0.88 g. NaOH/100 g dry cell) for 30 minutes. The samples were submitted for bread-baking test. The results are summarized in Table IV. The best result, as it is comparable to that of NFDM, is from the			
20	dough property. The baking test reducing the heat treatment.	7.5. The most significant improvement is in its esults indicate the importance of the pH effect	20	
	Performan	TABLE IV ce in Dough Handling		
	As Additive (2%)	Characteristics		
25	NFDM	Good in mixer, rounding, and moulder. Normal into oven.	25	
	Untreated Cells	Not tolerant to mixer. Sticky and stringy off mixer. Recovered for		
30	Cells treated* at pH 5.9 Cells treated* at pH 7.5	rounding. Flat into oven. The same as that of untreated cells. Equal to that of NFDM.	30	
	*Heating at 95°C. for 30 minu	utes under open air with constant agitation.		
<b>3</b> 5	groups. Soluble compounds such a group in the water soluble protein a structure by the sulfhydryl-disulfide and proofing. The thiol group is	reated yeast cells have a high content of thiol as glutathione and cystine, as well as the thiol re active materials which will weaken the gluten e interchange reaction during the dough mixing readily oxidized, especially under heating at	35	
40	increased pH with trace amounts of metal ions. Experimental results in Table V illustrate the effect of heating at increased pH in order to achieve the auto-oxidation of thiol in Candida utilis cells. Two things are indicated: (1) the thiol may be oxidized to various compounds beyond the less oxidized form of disulfide as			
	auto-oxidation from heating at the 5.9, and (2) almost all of the remains	61% of the total thiol in yeast is lost through the pH of 7.5, while only 30.5% is lost at the pH of aining thiol groups are in reactive form which		
45	apparently represents the thiol gro and unreacted. These residual the probably inactive during bread-ma	ups of insoluble protein existing intracellularly iol groups in the treated yeast cell are most king when the cells are mixed into the dough, as glutathione and cystine will affect the gluten	. 45	

TAB	LE V		

	Effec	t of Heating at	TABLE V Increased nH o	on the Auto-oxidat	ion of	
		T	hiol in Yeast C	ells <sup>(1)</sup>		
5	Cell Treatment	Reactive S (milli-equival gram)	ents/ (1	Total SH nilli-equivalents gram)	SH Loss	5
	Untreated pH 5.9 <sup>(2)</sup> pH 7.5 <sup>(2)</sup>	21.6 12.9 10.4		30.7 21.3 12.0	0 30.5 61.0	
10	(1) Candida utilis ATCC 9256. Continuous culture grown on ethanol at O <sub>2</sub> —limiting condition. (2) Heating at 95°C. for 30 minutes under open air with constant agitation. (3) Analyzed by the method of C. C. Tsen and J. A. Anderson ("Determination of Sulfhydryl and Disulphide Groups in Flour and Their Relation to Wheat					10
15		Cereal Chem. 40			nation to wheat	15
	pH 7.0 for one	hour. The baking	g test results as	I torula yeast cell slu summarized below e to bread-baking.	indicate that its	
20		mple	Bread Score	Dough Property	<u>.</u>	20
	Untreat Treated NFDM	cell	83 97 98	sticky and wet normal normal		
0.5			EXAMPLE I			
25	Aliquots of dispensed into e calcium compo	f 200 ml of year each of the 400 r ounds as listed	st cream which nl beakers, with in Table VI.	carried out as outli contains 10% cel or without the ad The amount of cather aliquot of cell i	l by weight, are dition of various alcium added is	25
30	ratio of calciun The slurry constant stirring quickly cooled	n to phosphorus was heated rap g. At the end of l down to room te	of one. pidly to 80°C. 0 minutes' cook mperature by ci	by a submerged s sing period, the hea reulating the cooling ared and adjusted, a	team coil under ted material was ng water through	30
35	value of 6.7. Th was directly sub treatment using summarized in	e cell material w jected to sensor	as separated, w y test without fi m compounds and VIII.	ashed, and dried. The astronomy as compared to	The yeast extract he results of the	35
40	1. A bland where the pH i reacted with Carre 2. The year	taste cell mater s close to the ne a(OH) <sub>2</sub> at an all ast extracts obt	ial is obtainable utral. Bad flavo caline pH, or w ained from the	by cooking the years are produced writh CaCl <sub>2</sub> at an ace treatment with blor, odor, and taste	hen the cells are idic pH. various calcium	40
45	control. The be 3. Tasting treatments indi	est choice is still of the unfraction cated that calcion means that a co	the one from nated products um carbonate	the CaCO <sub>3</sub> treatm prepared from th (CaCO <sub>3</sub> ) treated n se to 7 is very critic	ent. e above calcium naterial gave the	45

good structure

Gray color, gummy

55

(treated according to

Example X)

(untreated)

55

80% Triticale Flour 20% inactive dry yeast

<sup>\*</sup>Yellow cake score. Maximum possible score is 100 for best overall quality. The score for yellow cakes with 100% egg ranges from 94 to 96.

mixtures of single-cell protein with plant protein, whey solids, or both

8	1,575,052	88
5	plant protein and whey solids, said mixtures containing from 1 to 99 weight percent of the single-cell protein component; (b) heating the aqueous slurry to a temperature of from 75° to 100°C; (c) adjusting the pH of the heated slurry to within the range of 6.6 to 8.0 by adding a compound selected from anhydrous ammonia, ammonium hydroxide, calcium hydroxide, sodium hydroxide, sodium bicarbonate, calcium sulfate, potassium carbonate, calcium carbonate, sodium carbonate, potassium hydroxide, magnesium hydroxide and mixtures	5
10	thereof; (d) maintaining the heated, pH-adjusted slurry at said conditions for a time period of from 1 to 120 minutes; and (e) drying the material from step (d).	10
15	<ol> <li>The process of Claim 1 wherein the protein-containing material in step (a) is a mixture of yeast and whey.</li> <li>The process of Claim 2 wherein the aqueous slurry is maintained at a pH of 7.0—7.6 for from 2 to 4 minutes.</li> <li>The process of Claim 3 wherein the aqueous slurry is maintained at about</li> </ol>	15
20	<ul> <li>80°C.</li> <li>5. The process of Claim 4 wherein the pH is adjusted by adding calcium carbonate and calcium hydroxide.</li> <li>6. The process of Claim 1 wherein the protein-containing material in step (a) is a mixture of yeast and whey and the aqueous slurry is maintained at a pH in the</li> </ul>	20
25	range of 6.8 to 7.0.  7. The process of Claim 6 wherein the aqueous slurry is heated to about 80°C. for from about 2 to about 4 minutes.  8. The process of Claim 7 wherein the pH is adjusted by adding sodium hydroxide.	25
30	9. The process of Claim 1 wherein the protein-containing material in step (a) is plant protein material.  10. The process of Claim 9 wherein the plant protein material is a soybean material.  11. The process of Claim 10 wherein the aqueous slurry of soybean material is	30
35	maintained at a pH of from about 6.5 to about 7.5 for about from 30 to about 60 minutes.  12. The process of Claim 11 wherein the pH is adjusted by the addition of calcium carbonate and calcium hydroxide.  13. The process of Claim 12 wherein the slurry is heated to about 90°C.  14. The process of Claim 13 wherein an amino acid is added to the slurry prior	35
40	to heating.  15. The process of Claim 14 wherein the amino acid is methionine or cystine.  16. The process of Claim 1 wherein the protein-containing material in step (a) is a mixture of yeast and defatted soy flour.  17. The process of Claim 1 wherein the protein-containing material in step (a)	40
<b>45</b>	is a mixture of yeast and full fat soy flour.  18. The process of Claim 1 wherein the protein-containing material in step (a) is a mixture of yeast and triticale flour.  19. A process for improving the functional properties of a yeast material comprising the steps of:	45
50	a) preparing an aqueous slurry of the yeast material; b) heating the slurry to a temperature of from 75 to 100°C.; c) adjusting the pH of the slurry to from 7.2 to 7.6; d) maintaining the heated, pH-adjusted slurry at said temperature and pH for from 1 to 10 minutes; and	50
55	e) drying the slurry.  20. The process of Claim 19 wherein the yeast is Candida utilis.  21. The process of Claim 19 wherein the slurry is heated to about 80°C.  22. The process of Claim 19 wherein the pH is adjusted by the addition of calcium hydroxide and calcium carbonate.	55
60	23. The process of Claim 19 wherein the slurry is maintained at said temperature and pH for about 2 minutes.  24. A process for improving the functional properties of Candida utilis yeast comprising the steps of:  (a) preparing an agueous slurry of the yeast material;	60
65	(b) treating the slurry by maintaining the slurry at a pH in the range of 7.2 to 7.6 and a temperature of about 80°C. for about 2 minutes, wherein the pH	65

	is adjusted by the addition of calcium hydroxide and calcium carbonate; and	
	(c) drying the treated slurry.	
	25. A process for improving the functional properties of protein-containing	
5	materials, comprising the steps of:	5
•	(a) preparing an aqueous slurry of a protein-containing material selected from	•
	(1) single-cell protein, (2) plant protein, (3) whey material, and (4)	
•	mixtures of single-cell protein with plant protein, whey solids, or both	
	plant protein and whey solids, said mixtures containing from 1 to 99	
10	weight percent of the single-cell protein component;	10
	(b) heating the aqueous slurry to a temperature of from 75° to 100°C.;	
	(c) adjusting the pH of the heated slurry to within the range of 6.6 to 8.0 by	
	adding a compound selected from anhydrous ammonia, ammonium	
	hydroxide, calcium hydroxide, sodium hydroxide, sodium bicarbonate,	
15	calcium sulfate, potassium carbonate, calcium carbonate, sodium	15
	carbonate, potassium hydroxide, magnesium hydroxide and mixtures	
	thereof;	
	(d) maintaining the heated, pH-adjusted slurry at said conditions for a time	
ań	period of from 1 to 120 minutes;	20
20	(e) separating the slurry into a protein extract and a residual base-treated protein material; and	20
	(f) washing the residual protein material with water and drying same.	
	26. The process of Claim 25 wherein the protein-containing material is a yeast.	
	27. The process of Claim 26 wherein the yeast is Candida utilis.	
25	28. A process for improving the functional properties of protein-containing	25
	materials according to Claim 1 and substantially as hereinbefore described and	
	exemplified.	
	29. The product obtained by a process according to any preceding claim.	

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COMPLETE SPECIFICATION

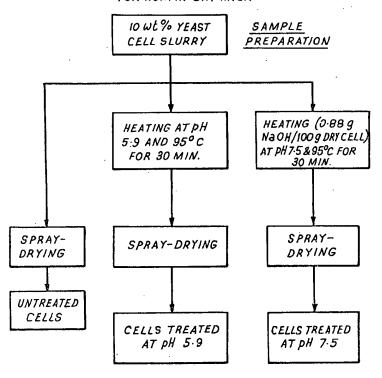
3 SHEETS

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Sheet 1

FIG. 1

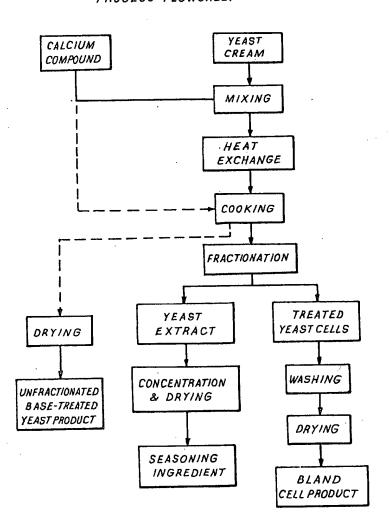
COMPARATIVE PROCESSES FOR PREPARING A YEAST, PLANT
OR YEAST-PLANT PRODUCT TO BE USED AS A REPLACEMENT
FOR NONFAT DRY MILK



COMPLETE SPECIFICATION

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FIG. 2
PROCESS FLOWSHEET



COMPLETE SPECIFICATION

3 SHEETS

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FIG. 3
PRODUCTION OF MODIFIED PLANT PROTEIN

